Amendments to the Claims

This listing of claims will replace all prior versions, and listings of claims in the application.

Listing of Claims:

1-8. (canceled)

9. (currently amended) The method of claim 47 ~~11~~, wherein the subcellular compartment ~~defined area~~ is selected from the group consisting of a ~~the~~ cell nucleus, a cytoplasm, a nuclear membrane, a cellular membrane, a mitochondria, an endoplasmic reticulum, a peroxisome and a lysosome.

10. (currently amended) The method of claim 47 ~~11~~, wherein the biomarker is selected from the group consisting of a protein, a peptide, a nucleic acid, a lipid or a carbohydrate.

11-38. (canceled)

39. (currently amended) The method of claim 47 ~~11~~, wherein each of the first, the second and the third ~~the~~ stain comprises ~~is~~ a fluorophore.

40-46. (canceled)

47 (new) A computer implemented method for localizing and quantitating a particular biomarker within a plurality of subcellular compartments present in individual cells of interest contained in a tissue sample comprising:

a) incubating the tissue sample with a first stain that specifically labels a first marker defined subcellular compartment, a second stain that specifically labels a second marker defined subcellular compartment, and a third stain that specifically labels the biomarker;
b) obtaining a high resolution image of each of the first, the second, and the third stain in the tissue sample using an upright or inverted optical microscope so as to obtain:
    i. a first image of the first marker defined subcellular compartment;
    ii. a second image of the second marker defined subcellular compartment; and
    iii. a third image of the biomarker;

wherein each image comprises multiple pixel locations;
c) reiteratively analyzing each pixel location in the first and the second image so as to assign each such pixel location to the first, the second or neither subcellular compartment based upon an intensity value of the first stain relative to the second stain at that pixel location;

d) analyzing in the third image the pixel locations assigned to the first or the second subcellular compartment in step (c) so as to identify those pixel locations having an intensity value indicative of the third stain, and determining the total intensity value of the third stain at the pixel locations in each of the first and the second subcellular compartment;

so as to thereby localize and quantitate the biomarker in the first or in the second subcellular compartment.

48. (new) The method of claim 47, wherein the quantitation of the biomarker present within the first or the second subcellular compartment comprises summing the intensity values of the third stain at the pixel locations within such subcellular compartment and dividing the sum by the number of pixels in such subcellular compartment.

49. (new) The method of claim 47, wherein a pixel location not assigned to the first or the second subcellular compartment is assigned to a third subcellular compartment.

50. (new) The method of claim 47, wherein the tissue has a thickness of about five microns.

51. (new) The method of claim 47, wherein the high resolution image comprises 1024 x 1024 pixel locations.

52. (new) The method of claim 47, wherein the first subcellular compartment is a cellular membrane and the second subcellular compartment is a cell nucleus.

53. (new) The method of claim 47, wherein the tissue sample is a fixed tissue section.

54. (new) The method of claim 47, wherein the first or the second stain reacts with a marker that is selected from the group consisting of cytokeratin, beta catenin, alpha catenin and vimentin.

55. (new) The method of claim 47, wherein at least one of the first, the second or the third stains comprises a fluorophore selected from the group consisting of 4',6-diamidino-2-phenylindole (DAPI), Cy3 and Cy-5-tyramide.

56. (new) The method of claim 47, wherein the biomarker is selected from the group consisting of Her-2/neu, estrogen receptor, progesterone receptor and epidermal growth factor receptor.

57. (new) The method of claim 47, further comprising after step (b) but before step (c) performing a pseudo-deconvolution step comprising:

1. obtaining an out-of-focus image of each of the first, the second and the third stain in the tissue sample wherein each image has an out-of-focus intensity value for each pixel location; and
2. subtracting the out-of-focus intensity value for each pixel location from the intensity value at such pixel location in the first, the second and the third images of step (b);

so as to thereby obtain a processed image for each stain, corrected for background.

58. (new) The method of claim 47, wherein a mask is applied to the first, the second and the third images.

59. (new) A computer implemented method for localizing and quantitating a particular biomarker within a plurality of subcellular compartments present in individual cells of interest contained in a tissue sample comprising:

a) incubating the tissue sample with a first stain that specifically labels a first marker defined subcellular compartment, a second stain that specifically labels a second marker defined subcellular compartment, and a third stain that specifically labels the biomarker;
b) obtaining a high resolution image of each of the first, the second and the third stain in the tissue sample using an upright or inverted optical microscope so as to obtain:
    i. a first image of the first marker defined subcellular compartment;
    ii. a second image of the second marker defined subcellular compartment; and
    iii. a third image of the biomarker;
wherein each image comprises multiple pixel locations;
c) (1) determining the first and second stain intensity in each of the pixel locations in the first and the second image and assigning those pixel locations having an intensity indicative of:
    i. the first stain only, to the first compartment;
    ii. the second stain only, to the second compartment;
    iii. both the first and the second stain to the compartment for which the stain intensity is greater or to neither compartment if the stain intensity is substantially equal;
(2) reiteratively analyzing the first and the second stain intensity in each of the pixel locations assigned to the other compartment to assess spillover and reassigning each pixel location based on a weighted ratio of first to second compartment intensity to reach a 95% degree of accuracy in the assignment;
d) analyzing in the third image the pixel locations assigned to the first or the second subcellular compartment in step (d) so as to identify those pixel locations having an intensity value indicative of the third stain, and determining the total intensity value of the third stain at the pixel locations in each of the first and second subcellular compartment;

so as to thereby localize and quantitate the biomarker in the first or in the second subcellular compartment.